ImmunoTools *FlowISiAM* Award 2024

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Evaluating the diagnostic and prognostic potential of the *FlowISiAM* assay in the peripheral blood of patients with multiple myeloma

Background: Multiple Myeloma (MM) is the second most common hematologic malignancy, characterized by the accumulation of neoplastic plasma cells in the bone marrow (BM) and the release of excess monoclonal immunoglobulin (M-protein) in blood and urine [1]. MM derives from precancerous entities termed Monoclonal Gammopathy of Undetermined Significance (MGUS) and smoldering MM (sMM). MGUS occurs in 3-4% of the total population over the age of 50 and describes a benign condition characterized by M-protein and accumulation of <10% clonal plasma cells in the BM. sMM represents an intermediate stage between MGUS and MM and reflects higher levels of M-protein and higher BM infiltration than MGUS [2].

The current prognostication systems are helpful in the clinical management of active MM, however they lack of a biomarker reflecting tumor burden, which could probably improve patients' risk stratification and help in defining the most appropriate treatment option [3]. One of the main challenges in estimating the actual myeloma burden is the patchy nature of the disease, where MM is unevenly distributed throughout the BM. Moreover, extramedullary involvement is also common and may occur in about 8-10% of newly-diagnosed MM (NDMM) patients [4]. Therefore, a BM aspiration from a single site, which is the standard method for diagnosis and monitoring of MM, has raised serious concerns regarding its representativeness of the overall disease burden [5]. In this context, liquid biopsy offers an alternative and invasive-free examination for assessing disease burden, through the

evaluation of circulating tumor cells (CTCs) or other biomarkers detected in the circulation (e.g. cell-free DNA) from various tumor sites and thus, reflects tumor load as a whole.

Recently, we and others provided evidence that higher levels of CTCs are independently associated with worse clinical outcomes in NDMM patients and could be utilized to refine current prognostication [6-8]. However, there seems to be a moderate correlation between CTC numbers and BM infiltration, and no study has correlated the differential presence of CTCs with extramedullary disease. In this project, we will investigate the actual disease burden of NDMM using the *FlowISiAM* technology and evaluate its usefulness as a non-invasive prognostic tool and as a biomarker of various infiltrated sites within and outside the BM.

Experimental Design and Methods: The analysis of liquid biopsy with the *FlowISiAM* technology will be performed both on fresh peripheral blood (PB) samples at diagnosis, as well as material derived from our biobank (comprising >1000 PB samples). Samples used from our biobank are accompanied by clinical information regarding demographics, BM infiltration, disease stage by established prognostic scores, CTC levels, imaging data including the presence/absence of extramedullary disease (evaluated by MRI/PET scans) and patients' outcome (response to treatment, time to next progression, etc). PB samples from healthy individuals, patients with MGUS or sMM will be used as controls indicating no, low, and a moderate disease burden. Moreover, clinical samples at the stage of diagnosis from patients with various solid tumors (e.g. breast, colon, lung) will also be available, as additional positive controls of the *FlowISiAM* testing.

Impact: The proposed investigations are expected to provide a non-invasive tool for a sensitive estimation of the actual disease burden in NDMM patients. On clinical grounds, the establishment of a competent myeloma burden indicator will provide multiple benefits, including the refinement of current patients' stratification systems, the cost-effective replacement of invasive, painful, costly and sometimes unnecessary diagnostic examinations (e.g. BM aspiration, imaging testing) and also, can assist/guide the selection of the appropriate therapeutic option.

Cooperation Partner: Prof. Ourania Tsitsilonis and Dr. Ioannis Kostopoulos will work together with ImmunoTools to adjust the experimental and instrumental set-up in order to conduct the *FlowISiAM* analysis. ImmunoTools and it´s partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test outcomes. Moreover, Immunotools will support the project by providing antibodies to be added to the already established flow-cytometric panel for detecting clonal cells, that together with the *FlowISiAM* testing will enable the estimation of the actual disease burden.

References

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